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PROBLEMS OF HEAT STERILIZATION DYNAMICS

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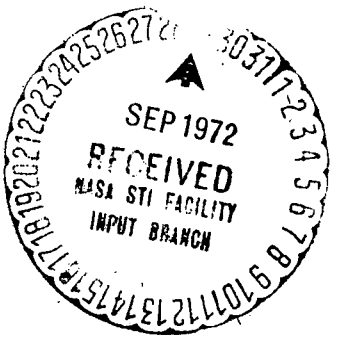

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PROBLEMS OF HEAT STERILIZATION DYNAMICS

Sadao Komemushi

ABSTRACT. Death behavior of microorganisms during heat sterilization is studied in connection with conformity or non-conformity to the logarithmic death law. Various interpretations of the dynamics of the logarithmic death law are cited from the literature, and possible explanations for non-logarithmic death curves are advanced. The role of thermal activation phenomena in heat sterilization is discussed.

Introduction

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"Sterilization" is an indispensable process in the fermentation industry, the food-processing industry, and the pharmaceutical industry. There are unexpectedly few books dealing in general terms with the all-important methods of sterilization and with related problems [1 - 4]. In most of them, the question is merely treated as one part of a number of unit operations (processes). Various sterilization methods are in use. Here, let us consider, from the standpoint of dynamics, the problems connected with the "heat sterilization method."

In his definition of the "dynamics of heat sterilization," Terui (1968) [5] states: "in the narrow sense, it is the dynamics of thermal death of groups of microorganisms. However, in view of the actual meaning of the process of sterilization, if we interpret it in a broad sense, discussing the relationship between the heating process and the death behavior of the microorganisms which are the object of heat sterilization in a system consisting of these microorganisms and their medium, taking into account also questions of

*Numbers in the margin indicate pagination in the original foreign text.

the movement of heat within the medium, we will obtain what might be called 'heat sterilization engineering,' if we introduce into this target-elements concerning such matters as the quality of the medium."

Research on the dynamics of heat sterilization begins with the study of the dynamics in the narrow sense as defined above and extends as far as dynamics in the broader sense. However, two intermediate systems are conceivable in the process leading from one to the other:

i) Systems in which the heat movement time cannot be ignored;

ii) Systems in which quality degeneration of the suspension medium (and of the effective components suspended or dissolved therein) cannot be ignored.

Concerning the first type of system, the writings of Hirai (1968) [6], Nakano and Miyoshi (1963) [7], C.R. Stumbo (1965) [8], and J.W. Richards (1968) [9] summarize studies in which heat movement was taken into consideration in connection with the design of heat sterilization equipment, using sterilization methods for canned foods as the materials for study. Similar studies concerning the sterilizing conditions of fermentation culture media were also performed by F.H. Deindoerfer et al. [10 - 13], who have reported concerning methods of calculating the sterilizing conditions. Concerning systems of the second type, a comparison of the activation energy (obtained from the inactivation speed of microorganisms) with the activation energy of vitamins reveals that short-time sterilization in the high temperature zone has good effects in preventing quality degeneration of the effective components [14]. For this reason, in heat processing of milk, it is said that the HTST (high temperature short time) method and the UHT (ultra high temperature) method are more effective than pasteurization [15]. As the heat processing of milk has undergone these changes, passing from pasteurization through the HTST method to the UHT method, research has progressed quite close to the broader definition of dynamics as given above, and the milk-processing field is apparently one of the most advanced fields in the food-processing industry with respect to heat processing.

In this general survey, in order to simplify the question, let us interpret the "dynamics of heat sterilization" in the narrow sense of the term.

"Spores" (endospores of bacteria) have been used by many researchers in the study of heat sterilization due to their great heat resistance. Many studies have been made concerning the properties of spores, including their great heat resistance, which is unique to them alone [16 - 21]. In order to attain a rational method of heat sterilization, it is indispensable to undertake physiological studies of the objects to be sterilized (among which "spores" are of the greatest interest). Here, however, we shall not deal with this aspect.

1. Logarithmic Death Law of Microorganisms

When the nutritive cells or spores of many types of microorganisms are subjected to heat treatment, it is frequently found that the logarithmic curve of survival, which plots the logarithmic value (lnN) of the number of surviving bacteria at that time, has a linear relation to the processing time, t. This behavior is similar to the timing of the "single-molecular reaction" which occurs during chemical reactions, and the formula for it can be obtained by using the number of surviving bacteria N in place of the concentration of the unreacted substances in a chemical reaction. Thus, we obtain:

$$-\frac{dN}{dt}=kN \tag{2.1}$$

or

$$N=N_0\cdot e^{-kt}. \tag{2.2}$$

(Refer to Figure 1.) Here, N_0 is the number of survivors when the processing time is t = 0, and k is the "death rate constant" at this processing temperature. It is obtained from the following:

$$k=\frac{1}{t_2-t_1}\ln\frac{N_1}{N_2} \tag{2.3}$$

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Here, N_1 and N_2 are the numbers of survivors at processing times t_1 and t_2.

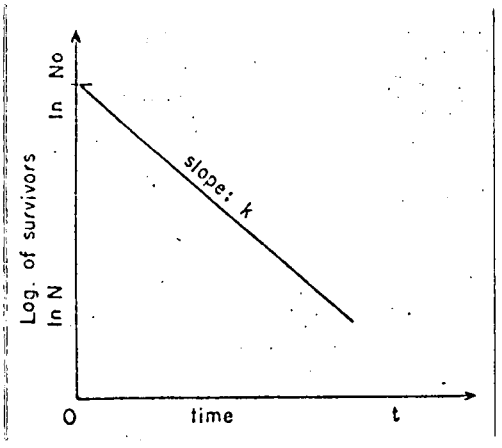

Figure 1. Logarithmic death of microorganisms.

A. Discovery of the Logarithmic Death Law. J. Lister, obtaining a hint from L. Pasteur's research concerning spontaneous generation and putrefaction, introduced into surgery the method of using carbolic water for sterilization. Subsequently, measurements were made of the sterilization coefficients for the nutritive cells and spores of many types of microorganisms with respect to carbolic acid and other types of disinfectants [22], but no time measurements were made. B. Kröning and T. Paul (1897) [23], using spores of Bacillus anthracis, made temporal and quantitative studies of their death caused by $HgCl_2$. K. Ikeda (1897) [24], analyzing the data of B. Kröning and T. Paul (1897) [23], proposed the following experimental equation:

$$\frac{N_1}{N_2} \times \frac{t_1}{t_2} = \text{constant} \tag{2.4}$$

This is the first equation concerning the logarithmic death of microorganisms. The logarithmic death of microorganisms when treated by chemicals was subsequently confirmed in the nutritive cells and spores of many other types of microorganisms by T. Madsen and M. Nyman (1907) [25] and H. Chick (1908) [26].

T. Madsen and M. Nyman (1907) [25] subjected B. anthracis spores to heat treatment at 100°C and 110°C and studied the time changes taking place. They mentioned that the thermal deaths of these spores followed the logarithmic death law, but satisfactory data could not be obtained because of the inexactness of the test methods used. H. Chick (1910) adopted a test method similar to that which we employ today. That is, he suspended the test microorganisms in hot water (temperature 47-54°C), made samplings with a sterilizing pipette after different periods of time had elapsed, and measured the number of survivors by means of plate culture. Using the nutritive cells of Bacillus

typhosus, Bacillus coli commune, Staphylococcus pyogenes albus, and Bacillus pestis, he confirmed the logarithmic death due to heat treatment.

B. Interpretation of the Logarithmic Death Law. After the discovery of the logarithmic death of microorganisms, numerous interpretations have been proposed for it. Normally, the thermal death of microorganisms occurs at a rate in direct proportion to the number of bacteria surviving at that time. This is exactly as if each of the single bacteria were behaving like a single molecule, and it is believed, for this reason, that the inactivation of a simple sensitive molecule inside each of the bacteria brings about the death of that bacterium. Of course, as O. Rahn (1934) [28] has shown, the actual substance of the simple sensitive molecule within the bacterium, fitting the conditions mentioned above, is a question which is still being studied in various quarters today in connection with the death mechanism, and it is unclear whether it may properly be called a simple molecule or not [29].

To explain the interpretations offered in the past for the logarithmic death law, let us divide them up into the following four interpretations.

(a) The reaction rate theory

This theory assumes that the death of a bacterium is the result of a chemical reaction inside the bacterium. Death is considered to be a process of thermal inactivation of one molecule inside the bacterium. The thermal death process at this time can be approximated as a first order reaction for the following reasons:

i) The molecular bonds are destroyed by direct activation of the molecules by means of thermal energy. In the chemical reaction theory, reactions of this type proceed as first order reactions.

ii) The reaction between the molecule inside the bacterium and oxygen. Under conditions of excess oxygen, such as during sterilization by dry heat,

the oxidation reaction is regarded as a first order reaction.

iii) Reaction between the molecule inside the bacterium, and water or steam. Just as in the oxidation reaction, if one of the reagents (in this case water) is present in a great excess, even though the reaction may be a second order reaction, it still proceeds as a first order reaction.

In such a case, when indicating the reduced speed equation for the unreacted molecule concentration C during the first order reaction, we can use the number of survivors in place of C, as has already been mentioned.

(b) The stochastic process theory

If the death rate constant k in the death reaction rate formula (equation 2.2) is defined as the "probability that a bacterium will die because of heating within a unit of time," death can be regarded as a sort of Markoff process in stochastic process theory. In this case, the probability that a given state will occur in any given bacterium will depend directly on its state at the time immediately preceding this. This can be expressed in a transition diagram such as that shown in Figure 2.

At this time, the transition probability matrix is as follows:

$$M = \begin{pmatrix} \text{transition probability from life to life} & \text{transition probability from life to death} \\ \text{transition probability from death to life} & \text{transition probability from death to death} \end{pmatrix} \tag{2.5}$$

$$= \begin{pmatrix} 1-k & k \\ 0 & 1 \end{pmatrix} \tag{2.6}$$

The higher-order transition probability matrix after time t can be indicated as follows: /708

$$M^t = \begin{pmatrix} (1-k)^t & 1-(1-k)^t \\ 0 & 1 \end{pmatrix} \tag{2.7}$$

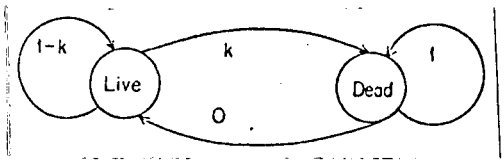

Figure 2. Transition probability diagram of microbial death.

Therefore, if the initial number of bacteria is N_0, the number of survivors after time t, N, will be:

$$N = N_0 \cdot (1-k)^t \quad (2.8)$$

$$= N_0 \cdot e^{-kt} \quad (2.9)$$

Thus, the logarithmic death rate can be obtained.

(c) Target theory

As was mentioned above, it was assumed that there was a simple sensitive molecule (the actual substance of which might conceivably be DNA, various types of RNA, protein, membranes, etc.) inside each bacterium, and that a fatal factor such as a radiation particle during radiation bombardment would collide with a target in the chemical structure of these substances. Death would result when the chemical structure was destroyed or altered as a result. As for the fatal factors in heat treatment, one need only assume something like the water molecules which are excited by thermal energy inside the cell, as has been proposed by S.E. Charm (1958) [30]. In the death pattern at this time, let us assume that k represents the probability that changes sufficient to make one target lose its normal functioning will occur as a result of collision between the target and the fatal factor within a unit of time. We can consider the number of targets in place of the number of cells. Then, if the initial number of targets is τ_0, the number of targets after time t will be:

$$\tau = \tau_0 \cdot e^{-kt} \quad (2.10)$$

If we assume that there is only one target present inside each cell, $N = \tau$, and $N_0 = \tau_0$. Therefore, equation (2.10) will be:

$$N = N_0 \cdot e^{-kt} \quad (2.11)$$

(d) Most probable lifetime distribution theory

Aiba and Toda (1965) [31], assuming a large number of cell groups, assumed that the heat-resistant life of the component cell units is determined before apparent heating, and derived a logarithmic death equation from the most probable lifetime distribution.

They established i classes of heat-resistant life from t_1 to dt_i. It was assumed that the numbers of cells belonging to each class were N_1, N_2, ..., N_i. The a priori probability that a unit cell would have a heat-resistant life t_i was defined as g_i. In this case, the probability of a heat-resistant life distribution $W(N_1, N_2, \ldots, N_i)$ will be

$$W(N_1, N_2, \cdots, N_i) = \frac{N_0!}{N_1!\ N_2! \cdots N_i!} \cdot g_1^{N_1} \cdot g_2^{N_2} \cdots g_i^{N_i} \tag{2.12}$$

Let us seek a combination of N_1, N_2, ..., N_i which will give the maximum value of $W(N_1, N_2, \ldots, N_i)$ in order to establish the mode of heat-resistant life distribution which would appear most easily. In this case, we obtain:

$$N_i = C_1 \cdot \exp(-\beta t_i) \tag{2.13}$$

In cases when the number of cells having heat resistant life t_i, N_i, displays a heat-resistant life distribution such as that shown in (2.13), $W(N_1, N_2, \ldots, N_i)$ will assume the extremal value (maximum value).

Equation (2.13) can be written continuously for cell groups with an infinitely large number.

$$-\left(\frac{dN}{dt}\right) = C_1 \cdot \exp(-\beta t) \tag{2.14}$$

Putting this into integral form, let us rewrite the coefficient. In this case, we obtain the following:

$$N = N_0 \cdot \exp(-kt) \tag{2.15}$$

In this case, k can be defined as the "inverse number of the average life until the reacting substance participates in the reaction."

In this manner, various interpretations of the logarithmic death law are possible. However, let us here assume that the death rate constant k is the "temporal probability density of occurrence of the event of death (hr^{-1})."

C. Metric Indications of Heat Resistance. Different researchers use different methods of indicating the heat resistance of microorganisms. Let us describe some typical methods.

(a) Thermal death rate constant (k)

This indicates the temporal probability density that the event of death will occur. H. Chick [27] sought this by:

$$k = \frac{1}{t_2 - t_1} \log(N_1/N_2) \quad (2.16)$$

Ordinarily, this value will not undergo changes in a series of heat sterilization experiments in which the initial bacterium concentration is varied. However, when Amaha (1952) [32] performed heat sterilization of spores of Bacillus natto at 100°C, the thermal death rate constant (k) assumed the following value on account of variations in the initial spore concentration (N_0):

$$k_1 = C_2 \times \left(\frac{1}{N_0}\right)^n \times \log_{10} N_0 \quad (2.17)$$

The cause of this is not clear, but it appears to be an interesting question (Figure 3).

(b) 90% death time (decimal reduction time)(D)

L.I. Katzin and L.A. Sandholzer (1943) [33] proposed the use of the heat processing time required to cause the death of 90% of a given microorganism group at a prescribed temperature. They proposed that this value be used as the "D-value." The relationship between the D-value and the thermal death rate constant k is:

$$D = 2.203/k \quad (2.18)$$ /709

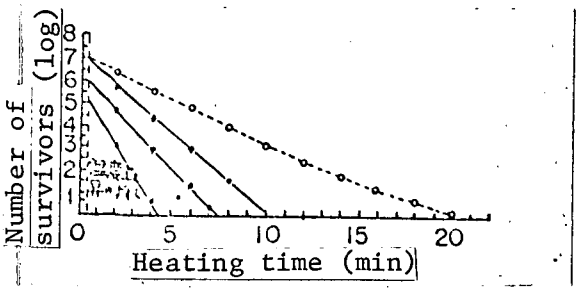

Figure 3. Survival curve of B. natto [32].

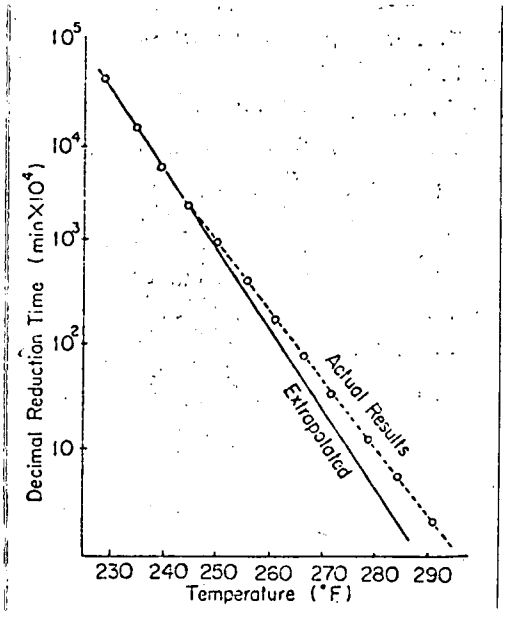

Figure 5. TDT curve of D. Wang, et al. [40].

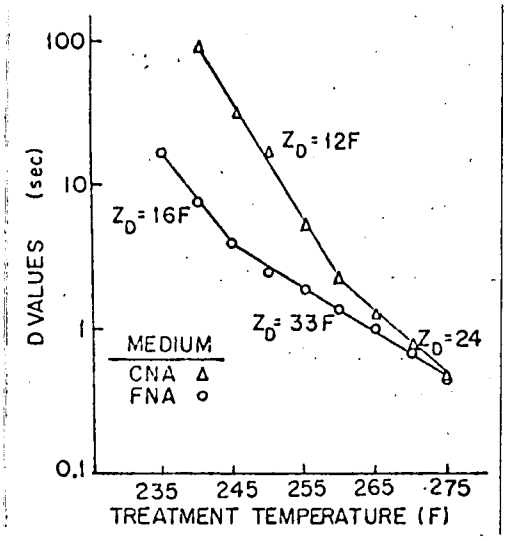

Figure 4. TDT curve of J.L. Edward, et al. [39].

In most cases, microbiologists use the D-value merely for the purpose of determining the time required in death, while technicians generally adopt k, since they are chiefly interested in the reaction rate.

Another value, in which the concept of this D-value has been further expanded, is the thermal reduction time (TRT). This coincides with the D-value at n = 1, defined as the time $(TRT)_n$ required to reduce a given group of microorganisms to 10^{-n} times.

$$TRT_1 = D \tag{2.19}$$

(c) Thermal death time (TDT)

The thermal death time (TDT) is the heating time required to cause death of all of a given group of microorganisms under definite conditions.

D. Bigelow and J.R. Esty (1920) [34] measured the thermal death time of spores of heat-resistant bacteria by a method using small test tubes. They showed that the TDT becomes a function of the temperature when the numbers of bacteria tested are identical. The TDT curve is the graph obtained by plotting the Log TDT (or the Log D) against the heat treating temperature.

The gradient of the TDT curve is called the Z-value, which has the following relationship with the temperature quotient Q_{10}, which is described below.

$$Z=10/\log Q_{10}(°C) \quad (2.20)$$

or

$$Z=18/\log Q_{10}(°F) \quad (2.21)$$

In sterilization of canned foodstuffs, etc., this value is an important parameter in calculating the sterilizing conditions. The TDT at a temperature of 250°F (121.11°C) is called the F-value. The TDT curve can be characterized by these F and Z values.

It is said that with many types of microorganisms the TDT curves will be straight lines both in wet heat sterilization [34 - 36] and dry heat sterilization [37, 38]. However, J.L. Edward, et al. [39], using spores of B. subtilis, report that in the high temperature zone, the curve assumes a tangential form with a convex shape at the bottom (Figure 4). D. Wang, et al. [40] report that the TDT curve of the spores of B. stearothermophilus does not have a linear shape in the high temperature zone (Figure 5).

During heat treatment in the high temperature zone, in comparison with that in the low temperature zone, the time required to raise the temperature of the spores which are to be sterilized to a given temperature and to cool them off becomes large enough that it cannot be ignored, as compared with the pure sterilizing time. It is probable that the TDT curve was believed to have a linear shape up to a rather high temperature zone because there was no

good method of handling the errors concerning this heat treating time.

(d) Thermal death point

The minimum temperature at which death of the test bacteria can be brought about after heat treatment for 10 minutes is called the thermal death point. Naturally, the value differs according to the quantity of test bacteria. /710

Among the metric methods of indicating the thermal resistance mentioned here, R and D are normally unrelated to the number of test bacteria, but TDT and TDP are values which change according to the number of test bacteria. Consequently, the former two values are suitable for theoretical studies of heat sterilization, while the latter are convenient when setting up actual sterilizing methods.

Various other metric indications of heat resistance, in addition to the four methods mentioned above, are also conceivable, but the other methods are not in very wide general use.

D. Relationship between Death Rate Constant and Temperature. S. Arrhenius [41] proposed the following equation for the relationship between the reaction rate constant and the temperature in a chemical reaction

$$\frac{d\ln k}{dT}=\frac{E}{RT^2} \tag{2.22}$$

Exactly the same equation applies to the thermal death rate constant. Integrating the above equation, we obtain the following.

$$k=A\cdot e^{(-E/RT)} \tag{2.23}$$

Here A is the frequency factor, and E is the activation energy of the reaction.

H. Eyring [42], using the concept of an active complex compound, derived the relationship between the reaction rate constant and the temperature from the absolute reaction rate theory.

$$k = \frac{k_B T}{h} \cdot \frac{F^{\ddagger}}{F_A F_B} e^{(-E_0/RT)} \tag{2.24}$$

In comparison with equation (2.23), there is a term here for the temperature in the portion corresponding to the frequency factor A, and it is believed that in a broad temperature range the values of A in equation (2.23) will undergo changes.

Equation (2.24) can also be altered as follows:

$$k = \frac{k_B T}{h} \cdot e^{-\Delta E^{\ddagger}/RT} \tag{2.25}$$

$$= \frac{k_B T}{h} \cdot e^{-\Delta H^{\ddagger}/RT} \cdot e^{\Delta S^{\ddagger}/R} \tag{2.26}$$

Here, $\Delta F^{\ddagger}$ is the free energy of activation, $\Delta H^{\ddagger}$ the activation heat, and $\Delta S^{\ddagger}$ is the activation entropy. Estimation of these thermodynamic variables will be of assistance in understanding the vital reactions which cause cell death.

The simplest method of indicating the relationship between the death rate constant and the temperature is the temperature quotient ($Q_{\Delta T0}$). Normally it is given in terms of the ratio between k_t at temperature t, and k_{t-10} at a temperature (t - 10), which is 10°K lower than t.

$$Q_{10} = k_t / k_{t-10} \tag{2.27}$$

Although this value varies with the temperature, it may be regarded as constant within a narrow temperature range. Therefore, it is frequently used. The relationship with the Z-value is exactly as described above. The value is 2 - 3 in ordinary chemical reactions, but it is known that there is a value close to 10 in cases such as the death of microorganisms or thermal degeneration of protein.

2. Non-Logarithmic Death Curves

In most cases, the survival logarithmic curves of microorganisms generally follow the logarithmic death law, but a large number of results have been reported which do not follow the logarithmic death law and which cannot be explained in terms of experimental error. If we classify the survival logarithmic curves in terms of their shapes only, we obtain the results in Figure 6.

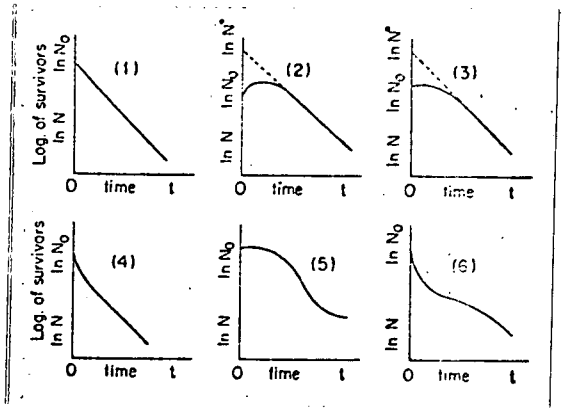

Figure 6. Type of survival curves.

(1) Logarithmic death pattern
(2) Increased number pattern
(3) Upper convex pattern
(4) Lower convex pattern
(5) (6) Composite patterns

The first pattern has already been discussed. As for the second, third, and fourth patterns, some efforts have been made to assign reasons to them or to provide analyses from the standpoint of dynamics.

A. Reasons for Non-Logarithmic Death Patterns. H. Chick [27] analyzed the thermal deaths of Staphylococcus pyogeneses aureus. He reported that the survival curves sometimes followed the logarithmic death pattern, and sometimes the non-logarithmic death patterns (3 or 4). He assumed that the cause was probably connected with the pre-culture history of the bacteria used. However, generally speaking, pattern 3 occurred in the newer ones, while patterns 1 or 4 appeared in the older ones. O. Rahn [28] also confirms similar findings.

According to F. Johnson, et al. (1954) [43], the exceptions to the logarithmic death law are attributable to the fact that it is impossible to make accurate measurements of the numbers of individual survivors in any processing time on account of the form of the bacteria used and the testing

conditions. Especially in cases when the bacteria used are types with lumpy shapes (Staphylococci, etc.), when they are joined in a chain shape (Streptococci, etc.), or when they are in a lumpy shape for some other reason, it is impossible to make accurate measurements of the number of survivors by the plate culture method. Nevertheless, he argues that experimental error is not the only reason and that there are also other factors, such as differences in the bacteria themselves in their susceptibility to death, the age of the culture, etc. In conclusion, he mentions the following five factors: /711

i) Precision of measurement of number of survivors

ii) Number of molecules which must be destroyed inside the cell when a living bacterium is changed into a dead bacterium (n in the multi-molecular model described below)

iii) Total number of such molecules inside a cell (m in the model below)

iv) Number of different types of molecules whose destruction results in death

v) Heterogeneity of the sensitivity of the individual bacteria.

C.R. Stumbo (1965) [44] discusses this problem from a different viewpoint and mentions the following factors as having an influence:

i) Thermal activation with respect to germination of the bacterial cell

ii) Admixture of a group with a different heat resistance

iii) Presence of a group of cells having a lumpy shape

iv) Cell agglutination during heating

v) Deagglutination during heating

vi) Conditions pertaining to the culture medium for counting the number of survivors.

Among these causes, let us next discuss the non-logarithmic thermal deaths which are not caused by manipulation factors and which are observed at times when the presence of heterogeneous groups or the influence of lumpy shapes are not conceivable.

B. Dynamic Analysis of Non-Logarithmic Deaths. Various death models have been proposed to explain the death behavior in patterns 2, 3, and 4 in Figure 6. There are some among them in which the derived values do not coincide with the test values, or in which the physiological significance of the parameters which have been assumed is unclear. However, let us now list the models which have been proposed thus far.

(a) Multi-molecular model

The second pattern of death behavior can be analyzed by this model. A system is assumed in which the death of all the cells occurs as a result of destruction of one type of molecule in the cells. It is assumed that the number of such molecules inside the cells is m, and that death occurs when n of these m molecules are destroyed. In this case, p is the probability that any molecule among the m molecules will be destroyed at any given time t. If q is the probability that this molecule will remain undestroyed, the following general equation will apply

$$1=(p+q)^m=p^m+mp^{(m-1)}q+\frac{m(m-1)}{2}p^{(m-2)}q^2+\left|\cdots+\frac{m!}{(m-n)!n!}p^nq^{(m-n)}+\cdots+q^m\right| \qquad (3.1)$$

The death rate depends on numbers m and n and on k, the rate constant for the reaction destroying the molecules. The probability of destruction of the molecules is

$$p = 1 - q = (1 - e^{-kt}) \quad (3.2)$$

On the other hand, the probability that the molecules will not be destroyed is

$$q = e^{-kt} \quad (3.3)$$

Generally speaking, in a (m,n) system in which death occurs because of destruction of n molecules among a total of m molecules, in equation (3.1), which has (m + 1) terms, the final n term is the one which gives the survival probability (Figure 7). That is,

$$(m, n) : N/N_0 = \sum_{i=0}^{n-1} {}_mC_i (e^{-kt})^{m-i} (1 - e^{-kt})^i \quad (3.4)$$

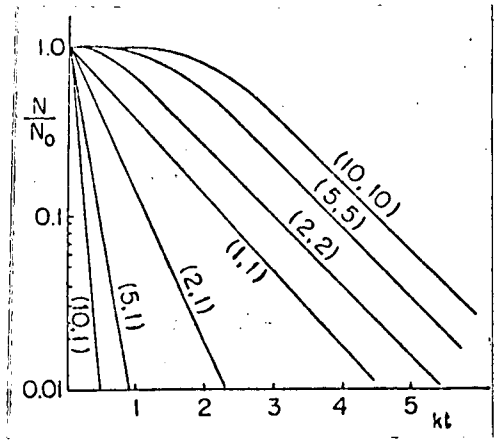

Figure 7. Theoretical curves illustrating the rate of death on multiple hit model.

Here, mCi is a binomial coefficient. The following equations are obtained as special cases of equation (3.4).

$$(m, m): N/N_0 = (1 - e^{-kt})^m \quad (3.5)$$

$$(m, 1) : N/N_0 = e^{-mkt} \quad (3.6)$$

$$(1, 1) : N/N_0 = e^{-kt} \quad (3.7)$$

W.A. Moats (1971) [45] assumed that death would occur among N critical sites when X_L of them were inactivated. In a system in which inactivation of the individual sites occurs at random, following the pattern of a first degree reaction, but the critical sites have exactly the same heat resistance, and the groups of bacteria are uniform in their heat resistance, he showed that death would follow a multi-molecular death model. He also described methods of calculating the various parameter values for this (k, N, and X_L). In addition, he sought the parameter values for Pseudomonas viscosa and Salmonella anatum.

As was pointed out above, if the values of m and n vary according to the amount of bacteria cultured, according to this theory there will be changes

in the death rate, even though the death rate constant k maintains a constant value [46]. A. Anellis, et al. (1965) [47] applied equation (3.5) to radiation sterilization of Clostridium botulinum spores (it is felt that the target theory applies best to them), and calculated the values of m. They found that the values differed even in agreement with the properties of the medium in which the spores were suspended. Furthermore, this theory becomes even more /712
complicated when the destruction of survival of more than one type of molecules has a relation to death.

(b) Two-line approximation model

H. Chick [27] used two lines to approximate the survival logarithmic curve in patterns 3 and 4. He obtained different death rate constants in the early and in the later period. M. Amaha and Z.J. Ordal (1957) [48] also used spores of Bacillus coagulans and obtained survival curves of the third pattern. Approximating these with two lines, they obtained D_1 and D_2 for the early period and for the later period.

A.E. Humphrey [14] and J.W. Richards [40] showed that when the initial microorganism group, rather than being purely homogeneous, is a mixed group with differing heat resistances, the survival logarithmic curves for each of the groups as they die in linear fashion will assume the form of pattern 4.

In the preceding two examples, it is inconceivable that two types of mixed samples which were totally different could have beenuused. Therefore, even though the two-line approximation method is a simple one, one cannot consider it to be a suitable analytical method.

(c) Initial number conversion method

One often obtains survival curves of patterns 2 and 3 in cases when thermal activation is necessary for germination of spores. In patterns 2 and

3 in Figure 6, let us extrapolate at t = 0 the logarithmic death curve for the later period of thermal sterilization. As a result, we will obtain the value N_0*. This model is one which attempts to express the later-period logarithmic death of the survival curve, assuming that

$$N = N_0^* \cdot e^{-kt} \tag{3.8}$$

In this model, when we have counted N_0 (viable), the number of survivors by plate culture when t = 0 (processing without heating), and N_0 (total), the total number obtained by microscopic examinations, it is obvious that

$$N_0 \text{ (viable)} < NN_0 \text{ (total)}$$

If thermal activation alone is the cause of non-logarithmic behavior in the initial period, we ought to obtain the following:

$$N_0^* = N_0 \text{ (total)} \neq N_0 \text{ (viable)}$$

However, it frequently happens that

$$N_0^* > N_0 \text{ (total)}$$

Thus, the significance attached to N_0* is vague, and this model is quite problematic.

(d) Activated spore model

A.E. Humphrey [14] mentions the analysis of survival curves of pattern 3 in connection with the death delay phenomenon which occurs in the initial period of heating upon thermal death of spores. That is, if k_1 is the rate constant for spores (N) becoming spores in the germinated state (N*), and also if k_2 is the rate constant for them to reach death, it will be possible to use the relationship

$$N = f(e^{-k_1 t}, e^{-k_2 t}) \tag{3.9}$$

and to make an approximate analysis of the survival curves of pattern 3.

J.J. Shull, et al. [50, 51] have made even more detailed studies of this same concept (Figure 8). Let us assume that the initial spore group is a mixed group consisting of activated spores (capable of forming a colony in a suitable culture medium) A_0 and non-activated spores (which are reversible and can be brought to the activated state by heating) N_0. In this model, the activated spores reach death by heating at rate constant k, but the non-activated spores die at k after having first changed to the state of activated spores (the rate constant of this thermal activation is α) (Figure 8).

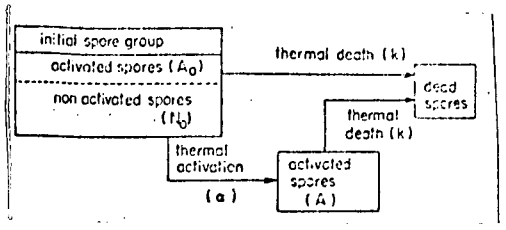

Figure 8. Activated spores model by J.J. Shull, et al. [51].

L_t, the number of live spores at time t, is

$$L_t = N_t + A_t \tag{3.10}$$

Here N_t is the number of non-activated spores at time t. It decreases by

$$N_t = N_0 \cdot e^{-\alpha t} \tag{3.11}$$

On the other hand, A_t is the number of activated spores at time t. The quantity of A remaining from the initial group is $A_0 \cdot e^{-kt}$.

The changes in A_t and L_t during a micro-time interval are as follows:

$$\frac{dA_t}{dt} = -kA_t + \alpha N_0 \cdot e^{-\alpha t} \tag{3.12}$$

$$\frac{dL_t}{dt} = -kA_t \tag{3.13}$$

When $\alpha \neq k$, the solution for (3.12) is

$$A_t = A_0 \cdot e^{-kt} + \frac{\alpha N_0}{k-\alpha}(e^{-\alpha t} - e^{-kt}) \tag{3.14}$$

When $\alpha = k$, the solution is

$$L_{t_2} - L_{t_1} = -k\int_{t_1}^{t_2} A_t dt \tag{3.15}$$

J.J. Shull, et al. [51] made comparisons of the test results with B. stearothermophilus and the results from the theoretical equation. They report that, although the curve shapes are similar, there are some differences. When attempts were made to correct these differences, the thermal activation ceased to follow the pattern of a first order reaction.

(e) Historical model

Terui (1966) [52] divided thermal sterilization systems into the following two main categories, depending upon whether the thermal sensitivity of the cells varied according to their thermal history during sterilization:

i) Nonhistorical system (the thermal prehistory during sterilization exerts no influence on the thermal sensitivity of the surviving cells)

ii) Historical system (the thermal prehistory during sterilization has an influence on the thermal sensitivity of the surviving cells) /713

He showed that survival curves of patterns 3 and 4 can be analyzed by using a model belonging to a historical system in which the value of k undergoes changes depending on the thermal history (Figure 9).

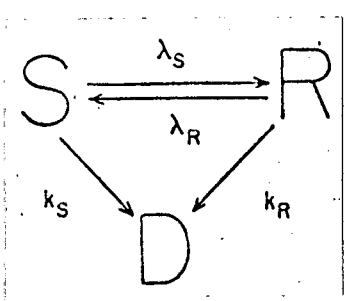

Figure 9. Model of simple historical system [52].

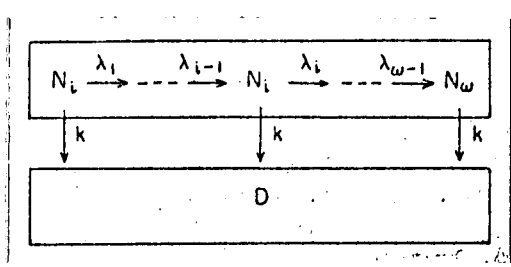

Figure 10. Schematic diagram of thermal inactivation [56].

The simplest type of historical system is a model of the single-molecular reaction pattern, in which the sensitive molecule upon being heated changes

from a low-sensitivity molecule (R) into a high-sensitivity molecule (S), or vice-versa. In either case, the molecule can become a degenerated molecule (D).

In the model in Figure 9, in pattern 3, $\Delta F_{R \rightarrow S} \ll 0$ under heating conditions, and in pattern 4, $\Delta F_{S \rightarrow R} \ll 0$. Here, k is the rate constant moving towards D, and λ is the rate constant for the changes between S and R. The suffixes indicate the points of departure for the reactions.

Following this model, let us formulate the equations according to rate theory. The following equations can be obtained.

$$N/N_0 = K_S e^{-(k_S+\lambda_S)t} + (1-K_S)e^{-k_R t} \quad (3.16)$$

$$N/N_0 = K_R e^{-(k_R+\lambda_R)t} + (1-K_R)e^{-k_S t} \quad (3.17)$$

$$K_S = \gamma_S(k_S-k_R)/(k_S+\lambda_S-k_R) \quad (3.18)$$

$$K_R = -\gamma_R(k_S-k_R)/(k_R+\lambda_R-k_S) \quad (3.19)$$

$$\gamma_S = 1-\gamma_R = N_S/N_0 = 1-\frac{N_R}{N_0} \quad (3.20)$$

Here, N_S and N_R are the initial numbers of S and R spores. γ_S and γ_R are the initial presence ratios of S and R. Furthermore, equation (3.16) corresponds to pattern 4, while equation (3.17) corresponds to pattern 3.

This model has been used to analyze the thermal death behavior of spores of B. subtilis var. niger and B. pumilus, which display survival curves of patterns 3 and 4 [35 - 55]. An interesting point is the nature of the reaction at the λ stage according to the estimated activation energy value. At the λ_S stage of B. pumilus spores, a protective reaction (S $\rightarrow$ R) occurs with respect to heat, and the activation energy is approximately 25 kcal/mole. On the other hand, in the case of the spores of B. subtilis var. niger, at the λ_R stage, a thermal activation reaction (R $\rightarrow$ S) takes place, and the activation energy is approximately 63 kcal/mole. The magnitude of the activation energy corresponds to that of thermal degeneration of protein. This point differs from the λ stage of B. pumilus.

Analysis of the above has been performed, assuming that both γ_S and γ_R have values of 1. In this model, the same sort of analysis can be performed when both S and R are present in the initial spores.

(f) Thermally damaged spore model

K. Toda (1970) [56] assumed the existence, in the heat treatment process, of thermally damaged spores, which were an intermediate state of the undamaged spores. He sought the changes in the number of spores in each of these states.

Let us consider the model in Figure 10 as the general pattern of the thermally damaged spore model and seek the rate of change in the number of spores at activation state "i."

$$dN_i/dt = -(k+\lambda_i)N_i + \lambda_{i-1}N_{i-1} \tag{3.21}$$

Here,

$$\lambda_0 = \lambda_{w+1} = 0 \tag{3.22}$$

The solution of N_i is

$$N_i = \sum_{j=1}^{i} A_j \cdot e^{-(k+\lambda_j)t} \tag{3.23}$$

Here, coefficient A_j is

$$A_j = \left(\prod_{j=k}^{i} \frac{\lambda_{h-1}}{\lambda_h - \lambda_j}\right)\left\{\sum_{\beta=1}^{i} B_{\beta j} N_{\beta 0}\left(\prod_{j=\beta}^{i} \frac{\lambda_r}{\lambda_j - \lambda_r}\right)\right\} \tag{3.24}$$

Coefficient $B_{\beta j}$ in equation (3.24) is a sign indicating function:

$$B_{\beta j} = \frac{1}{2}\left[(-1)^{\beta+1}\{1-(-1)^j\} + (-1)^{\beta}\{1 + (-1)^j\}\right] \tag{3.25}$$

The total number of live bacteria at time t is the total of N_i:

$$N = \sum_{i=1}^{w} N_i = N_0 \cdot e^{-kt} \tag{3.26}$$

The model shown in Figure 11 is a simple model derived from this general theory, by which it is possible to explain the thermal death process of B. subtilis spores. In this model, the initial spores are of two states:

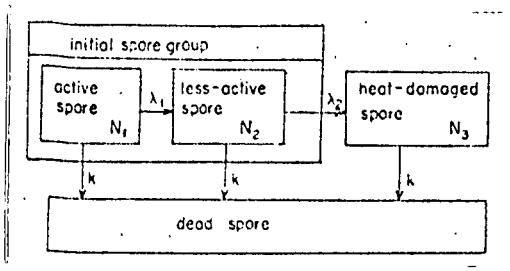

Figure 11. Schematic diagram of simpler model [56].

N_1 and N_2. This corresponds with the fact that the total number of surviving spores from the B. subtilis spores ($N = N_1 + N_2 + N_3$) displays a logarithmic death pattern, while the number of undamaged spores ($N^* = N_1 + N_2$) displays a survival curve following pattern 4. The values of N and N* at time t are given by the following equations:

$$N = N_1 + N_2 + N_3 = N_0 e^{-kt} \tag{3.27}$$

$$N^* = N_1 + N_2 = \frac{\lambda_2}{\lambda_2 - \lambda_1} N_{10} e^{-(\lambda_1 + k)t} + N_{20} - \frac{\lambda_1}{\lambda_2 - \lambda_1} N_{10} e^{-(\lambda_2 + k)t} \tag{3.28}$$

/714

Here, $N_0 = N_{10} + N_{20}$, $N_{30} = 0$.

In this model, the heat treatment processes of the spores can be explained in the following manner:

i) At least two types of activity distributions are present in the group of spores which have not yet been subjected to heat treatment.

ii) There is a successive inactivation reaction between the three types of live spore states of activity, including the thermally damaged spores.

iii) It is possible to treat the process as a "composite reaction" accompanied by a death reaction from each state of activity.

(g) Others

In the foregoing, we have listed some of the models which have been reported thus far and applied to the death of microorganisms. However, numerous other models are also conceivable if considered simply as models.

K.J. Laidler (1958) [57] has given numerous models concerning the degeneration of protein. Most of them are also valid as models for thermal death of microorganisms.

The model in Figure 10 assumes a composite reaction. It is also possible to consider "successive reaction models" in which intermediate stages before death are considered, or "multisite models," in which numerous types of sensitive molecules are considered, and in which the destruction of any of the sensitive molecules would lead to death. At any rate, what must be considered in the future is the necessity of clarifying the key reaction in death and of producing a model centering around this reaction. In this respect, also, it is felt keenly that it is important to investigate the causes of death as well as the causes of heat resistance.

C. Thermal Activation. One frequently observes thermal activation phenomena when bacterial spores are used as material for heat sterilization experiments. In the general treatises of A. Keynan and Z. Evenchick (1969) [58] or of R.W. Berk and W.E. Sandine (1970) [59], it is demonstrated in detail that thermal activation plays a necessary and important role in germination of bacterial spores. For this reason, the survival logarithmic curve does not appear in its true form. Instead, the difference between the increase in the number of colony-forming cells as a result of thermal activation and the thermal death number appears as the apparent survival curve of the initial period. Thus, we sometimes see an increase in the apparent number of live bacteria, or the survival curves sometimes assume a convex shape at the top.

In order to analyze the death behavior, it is necessary to remove this sort of activation by studying the preheating conditions which will not result in death. This amounts to the same thing as removing, by a suitable method, the counting errors resulting from congelation or flocculation. It is necessary to obtain a correct survival curve.

As for the relationship between thermal activation moving towards germination and thermal activation moving towards death, there are still interesting questions left for solution in the future. The latter is a reaction towards susceptibility to heat and corresponds to changes of k. The activation energy of germination is approximately 20 kcal/mole [60], but in thermal activation towards germination, approximately the same value as in thermal degeneration of protein (approximately 72 kcal/mole) has been observed in B. megaterium spores [61]. As for the thermal activation towards death, the value has been reported to be approximately 62 kcal/mole [55]. However, it is not clear whether both of these thermal activations are a single process or not.

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